NNPDF-Funded Research Grant # 28

TITLE: The Role of HE1 in Cholesterol Trafficking
PROJECT INVESTIGATOR: Daniel S. Ory, M.D.

PERIOD: 8/15/2001 - 8/14/2003

PROJECT DESCRIPTION

Niemann-Pick type C (NPC) disease is an inherited disorder characterized by the accumulation of cholesterol in the cells of affected individuals, which results in degeneration of the brain, liver and spleen tissues. The disease is progressive and often fatal within the first two decades of life. On the basis of genetic studies, mutations in at least two different genes, NPC1 and HE1, cause NPC disease. Because of the similarities in the NPC disease that is caused by mutations in either of these genes, we propose that the NPC1 and HE1 proteins work closely together in the sorting and distribution of cholesterol within the cell. The goal of our project is to establish whether NPC1 and HE1 are located in the same compartments within the cell and potentially interact, to determine the structure of HE1 and to characterize the cholesterol binding properties of HE1. The HE1 protein had previously been identified as a major protein in human epididymal fluid. The porcine form of HE1 has been shown to specifically bind cholesterol (one molecule of cholesterol for each molecule of HE1) though it has no apparent sequence similarity with other cholesterol binding proteins. These cholesterol binding properties are consistent with a role for HE1 in the bulk movement of cholesterol, perhaps as a cholesterol carrier from one site in the cell to another. Alternatively, HE1 may serve a regulatory role, possibly as a cellular cholesterol sensor. We propose that binding of cholesterol to HE1 induces a change in the shape of the protein, which in turn regulates cholesterol trafficking in the cell. The purpose of this project is to examine the role of HE1 in the movement of cellular cholesterol. In Specific Aim 1 we will characterize the location of HE1 within the cell with respect to NPC1 using microscopy methods. We will also try to detect potential interaction between the NPC1 and HE1 proteins. In Specific Aim 2 we will perform studies to determine the structure of the cholesterol binding site of HE1. The cholesterol binding site on HE1 will be labeled with a cholesterol analogue, followed by identification of the regions of the HE1 protein to which the analogue has bound. We will also determine how tightly HE1 binds cholesterol, the specificity of binding and the number of cholesterol molecules bound per HE1 protein, as well as examine alterations in structure of HE1 that are induced by cholesterol binding. The studies outlined in this proposal will further our understanding of the critical role of HE1 in cholesterol metabolism. Examination of potential interactions between HE1 and NPC1, and characterization of the structural basis for and consequences of cholesterol binding by HE1 will help elucidate the molecular mechanisms involved in sorting and trafficking of cholesterol. Furthermore, these studies will open the way for structure-based drug design to manipulate cellular handling of cholesterol, and may assist in the development of therapies for NPC disease.

FINAL STATUS REPORT
Dated 8/5/2003

The goal of our studies is to understand the role of the NPC2 gene in cholesterol metabolism. Our initial studies focused on examining the location in the cell where NPC2 functions. We hypothesized that the NPC1 and NPC2 proteins functioned at the same step in a common pathway to metabolize low-density lipoprotein (LDL)-derived cholesterol. We showed in cultured human skin cells that the NPC2 protein localizes in part to vesicles that also contain the NPC1 protein, though the bulk of the NPC2 protein was found with lysosomal proteins. In addition, studies in our laboratory and that of other investigators using a variety of techniques have been unable to demonstrate direct interaction between the NPC proteins. Contrary to our original hypothesis, these findings are more consistent with a model in which NPC1 and NPC2 act sequentially in a common pathway to metabolize low-density lipoprotein (LDL)-derived cholesterol. To understand how NPC2 participates in maintenance of cellular cholesterol balance, we next examined the effect of absence of NPC2 protein on the regulation of cholesterol levels in the cell. We found in NPC2 mutant cells a failure to properly regulate both synthesis of cholesterol and uptake by the cell of lipoprotein-derived cholesterol (for example LDL particles). These defects were attributable to failure of the NPC2 mutants to appropriately shut down the regulatory machinery responsible for these activities, as well as stimulating pathways that help the cell eliminate excess cholesterol. Unexpectedly, we found that the defect in cholesterol regulation in both NPC1 and NPC2 mutants correlated with the inability of these cells to produce metabolites of cholesterol termed oxysterols. Moreover, we demonstrated that treatment with oxysterols reduces cholesterol accumulation in the NPC mutants. These findings indicate that both NPC1 and NPC2 are necessary for generation of these cholesterol metabolites, and importantly have implications for the treatment of NPC disease.

PUBLICATIONS:

http://www.jbc.org/cgi/content/abstract/278/28/25517